THE UNIVERSITY OF WISCONSIN
COLLEGE OF AGRICULTURE

Madison 6

DEPARTMENT OF GENETICS

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Dear Bruce:

Hail, wassail stout fellow, joyeux noel, etc. We miss you too: it would have been some party last night could we all have gotten together over our cups. Spike (Spicer) helps remind us of Merrie England, but 'ere too long you'll have him back too.

I'm sorry not to have ansered you sooner about your draft-- I find it difficult to say anything about it at this stage, except that it seems to cover all the facts. I'd rather not have gone at this, and would rather see your final draft, as it is only thate that various points of emphasis and fact can be seen to be criticized. Nort' hasn't said anything to me about his views on the paper: I expect you'll hear straight from him. I'll try to dig out what suggestions I can at this stage, as you've prodded Esther for them. First, thanks for the numerous cultures received on the 23d. I was most interested in SL-13 -14 (Are these supposed to be identical). I wondered whether there weren't some inconsistency between your transduction of a para A, and its supposed incompetence to be adsorbed. After looking at its very low efficiency of transinduction (by PLT22/2 and 22B/609 etc), I see there may be no inconsistency, but I haven't tried to measure phage adsorption yet. Fla I think may be better than F, although more cumbersome. F may already have built up some connotations re compatbility. Fla can be pronounced; Flg cannot. H_1 is probably easier than H_{sp} for typographical reasons, but this is no strong argument. I would agree on Fla_1 for 543, and will accede to your other definitions henceforth, except that I think it unwise to save Fla_2 for SL-13. If it does turn out to be isolocal with Fla_1, that will leave a gap at the very beginning of the series which is awkward in completing checkerboards, etc. I would classify SL-13 as provisionally Fla_1 on the basis of the common linkage to H_1, and give it a distinctive number later if necessary In fact, it would be psychologically advantageous <u>not</u> to give adjacent numerals to linked loci, as this often tends to evoke a speciously simple image of structure. I would assign Fla_2 to some locus definitely different from Fla_1. When it comes to numbering loci, I suggest the burden is to prove difference, though the shoe's on the other foot when it comes to assertions of allelism. Slo 's ok, but I think a more elegant prefix would be desirable. Have you any classical scholars in your pocket? Re phage-typing, fine. Unbeknownst to me, Edwards had been doing the same on the strains sent him, with consistent results. On basis of rhamnose fermentation alone, the parent of 534 has to be #157. #157 came from N25, which I know have, and is a typical "java" strain. The change b--1,2 must have been a sporadic mutation, but I am making some further tests on the genetic homologies of its $H_1{}^b$. A major difficulty is a j phase (z33?) which crops up now and then, and for which I have not yet gotten serum for further genetic analysis. Dave should have this in hand soon, however. I applaud your plans to study the tracks. I don't understand your remark on gp-1,2. Have I miscopied something? The experiment was dublin O --x typhimurium, not the contrary. All that needs be postulated is the substitution of $H_1{}^{gp}$ for $H_1{}^i$. I haven't tried $H_2{}^2$ --x dublin.

The adaptation of PLT22 to paraB is still unsettled--just haven't gotten round to a careful titration of "22B" grown back on typhimurium, though I've made the lysates.

If I can get round to it, I'll add a briefing on some recent experiments on lysogenization. I'd better get to your draft first:

Title: I like 'em shorter, viz. "Genetic analysis of Salmonella flagella", or genetic determination, control, ...

Ia. Norton and I will probably set to review and summarize the evidence for FA- phage. Our recent lysogenization expts, seem to clinch this, and there's more yet. You will have to make a lucid recap., but I would suggest not including other experimental details in the first draft. They can be added if there seems to be room.

Ibc, II b, ø. Check. Table would be all right; not improper to group them, anticipating later work.

IIc. Definitely. Its reputation should be restored.

How about some discussion of physical properties, structure of gelatin agar? (E.G. temperature response).

III check. Incidence of lysogenicity may depend on adaptation of phage to host. E.g,. with LT-2 and PLT22, it is very high; with PLT22 and 666, very low.

IVa. table here: always easier to refer back than forward. Gallinarum not known to be transinduced for any character.

V check. VI ck. VII ch. Hirsch's O mutant might be very good for this in view of its high rate of phase-variation.

X. Here is the first place my emphasis in thinking differs from yours. It would be premature to conclude that the linkages have any physiological significance: could be coincidence. Also, argument is historically reversed. The linked transduction was stumbled on before itcwas looked for. Perhaps the closest analogy in E. coli is V_1^p-V_1^r-V_1^s system (CSH '51). Very close linkage of "pseudoallales" is exceptional--your draft seems to give the opposite impression. Try lwoffing 548? p.11 line 6 ¶.2 Which 2 instances? "in the present instances" ? Criticism of 543 as possibly a "monophasic abortant" --what does it mean? What would you call ~~xxxxxxxxxx~~ sandiego --x 543: monophasic chester, sandiego, stpaul...? Are you asking the question, is 543 recently or simply derived from a typical b:12 or b:enx strain? K. can't tell you, but I'd be amused to hear his response. Most people will recognize what you mean if you write paratyphi B, not java. Edwards mentioned that it reacted with various paraB phages, but no simple pattern.

Ratio of b:i is variable with FA from different stocks, and is rather poorly defined as later swarms may show diffenent ratios from earlier. I would not go into this, but simply put ca. 5% for t-m --x 543. Might mention that Gal- marker of 666 rules out contam. to explain i's, as does their monophasic behavior.

Detailed report (mirror absorptions) on crucial strains would be desirable. Can also mention general confirmation by Edwards, but phrase this carefully (perhaps, "~~xxxxxx~~ cultures obtained in similar expts. by JL have been verified by PRE) to avoid implication that we felt independent verification of their findings was necessary. ~~xxxxxxxxxxxxxxxx~~

But you really can't hold me to a serious discussion of the draft, which is superb. Will you give me an opportunity to see the final paper? I promise to be most prompt.

Back to lysogenization expts: (I must have included some remarks with my shipment of cultures -Have you gotten these yet? Also, have just sent out some reprints of Z&L, and that review of which most of the punctuation is yours. Supplies are fairly tight, but suggestions on who might profitably use addn'l reprints are welcome). In my last letter, I mentioned a prelimin. expt. This has been repeated with a more satisfactory control: Gal+Fla+ was added in small numbers to Gal-Fla- plus FA(Gal+). Papillae were picked and scored as Fla+/- and lysogenic/sensitive, after purification. The transductions (Fla- Gal+) were 18 lysogenic : 3 sensitive; the insertions (Fla+ Gal+) came out 3 lysogenic : 43 sensitive. Esther has done an equally decisive experiment with lambda. In both cases, the multiplicity of phage was high, and it was difficult at first to give detailed explanation, except in the general terms that lysogenization is directly connected with transduction. The former has

yet to be well understood, but the following reasoning may be useful. The non-lysogenic survivors in these experiments can hardly be regarded as cells that have never been infected. Instead, they are likely to be part of the progeny of infected cells, and, often, sibs to cells that have either lysed or become lysogenic. In this connection, it is worth noting that Esther found that the transductions were not obviously mixed lambda+ and s, whereas most of the controls that showed any lambda were. What we are doing, then, is to fix on that part of the progenies which have had the best opportunity to become stably lysogenic by separating the transductions from the parent cells, and this accounts for the higher incidence of lysogenicity. In E. coli, this incidence was 100% in the transductions; about 2/3 in the rest of the population (a good deal of this is overestimated by reinfection, of course). I had been planning a similar experiment at lower multiplicities, and using antiserum to prevent reinfection, so that the limiting factor would be supposedly the amount of phage, but until the lysogenization aspect is better understood, this may not be meaningful. In addition, even undiluted serum is not entirely effective in preventing cross-infection from bacterial infective centers. The point of these experiments is, of course, to try to show that FA=phage not only with respect to the skins, but also the contents.

Another approach is now possible with the help of a "lytic variant" of PLT22, 22V, noticed casually on LT-2. 22V lyses almost completely (survivors mostly roughs, not lysogenic, so far), but LT2(22) is resistant (Cf. C and C' of Burnet and Lush 1936). Thus, if 10^8 PLT22 is added to 10^9 LT-2, followed 10 minutes later by excess 22V one gets nearly 10^8 survivors. The plating of the PLT22-infected LT-2 by itself gives an expected proportion of <u>contaminated</u>, rather than lysogenic colonies, in agreement with Esther and my previous expts. with lambda. So I do not think, unfortunately, that resistance to 22V requires the ultimate stable lysogenic state. (I note your mention of a student doing something similar-- we shall have to arrange to avoid unnecessary overlap.) This protection experiment is something I longed to do with lambda some time ago, especially when I thought of the "transformations" as a sort of distorted lysogenization, but I never could find a mutant lambda or other phage with the necessary properties. Lominski had something similar too, of course. 22V should make it possible to connect FA with particles with <u>protective</u> ability, which will in turn produce <u>lysogenic</u> survivors. If most of the transductions are preserved (after infection at low multiplicity with PLT22) while most of the rest of the population is destroyed by by 22V, this would again correlate phage particles as actual carriers of FA. (In this, I am contending with the counterhypothesis that FA is phage skins that have incorporated bacterial fragments instead of phage nuclei.) As 22V itself has a trace, though rather definite, tranducing activity, tested on lysogenic recepter strains, some elementary precautions are needed for the experiment, and these have delayed it momentarily. 22V seems to be temperate for SW666.

Some more data with UV: By very long exposures (20 <u>mins</u>., our sterilamp at 50 cm) lytic activity of FA(e.g. SW618) can be reduced over six decades, while FA is diminished less than 1. This permits transductions (Gal+) and plaques to be counted on the same plate. The former are not lysogenic. As I may have mentioned, the intent of these experiments was to dissociate Fla from H_1 in the linked transduction. It appears doubtful, however, that UV is reaching the genetic material at all. In addition, the proportion of b:i in SW618--x 666 varies as between the early and the late swarms, and cannot therefore be accurately measured. I do not understand this very well, the purified isolates have about the same motility afterwards. Either there is a difference in the time of initiation, or the initial differences in rates (polygenic linked modifiers?) are levelled by subsequent selection before the isolates can be purified. One expt. with X-ray was not very promising: 200,000 r left about 10% phage and 30% (+) FA. These doses are too large to make any thorough investigation feasible.

Larry has been doing some UV-induction (lwoff) experiments for me: LT2, LT22 do not look very promising, but the SW543 line, infected with 22B, seems to be working very well. Lwoffates titrate to about 10^{10}, have the same pattern of Gal+, Fla+ transducing activity as lytically grown phage.

On phase genetics, I have been temporarily stopped, waiting for some new cultures from Edwards which may be sufficiently stable. For good technical reasons, I need diphasics (like abony) of which H_1 is neither i nor e, and of which H_2 does not react with anti-1,2.... If you yourself happen to be acquainted with any such serotypes of which you have the knowledge that they are relatively stable in flagellar phase, I should appreciate them. (Perhaps Joan Taylormight--would you ask her? I've sent Edwards the specifications, suggesting bispebjerg, abortus-bovis (competent to absorb?), durban or other abony's.)I could use some of my own recombinants, and perhaps will, but this might not be regarded as cricket. One gets into some perplexities wondering how a "heterophasic" transduction will work out on my scheme.
I have not yet succeeded in getting i from i:1,2 --x 666, but have perhaps not tried enough. It is possible that H_1^{i*} is capable of functioning in the monophasic residual genotype of SW666. This could be tested by looking at the competence of the $(H_1^{i*}$ --x 666)i --x abony, where it would no longer function. Alternatively, H_1^{i*} may undergo sufficiently rapid variation to H_1^{i} in the 666 residue to allow the i phase to be detected, especially if Fla+ H_1^{i*} is immotile, and therefore strongly selected against.

On a visit to Boston last month, I had an excellent time with Dienes, and took some of his material back with me. I think I can now grow L-forms from Proteus without too much trouble, but quite large inocula are still required at each transfer. He has promised to send me some material of the same sort from Salmonella for some genetic experiments (e.g. Fla+ L --x Fla- Bact.) These LSs are entirely irrevertible, which is some slight advantage. It seems to be necessary to preserve the integrity of microcolonies of the L's to permit further growth: they either must make a surface film for themselves on booth, or have physical support, either agar at just the right concentration, or cotton fibers, or as seems to be working a film of collodion. Microscopically, they are wierd; I do not yet have any evidence of my own that they are genetically derived from the original bacteria, and am keeping an open mind on the whole business. The small size and growth habit almost suggests that the genetic equivalent of a bacterial cell is the L-colony, but this is a speculative fancy.

Word of your magnificent talk also reached here via a letter to Spicer.

Have you heard of Novick&Szilard's recent work on antimutagens?--you must have en route home. It would be fun (forvyou?) to try these on the Salmonella mutations (phase variation, Fla+..) In fact, there's still no evidence of any mutagenic effect on these factors, is there?

Esther and I have been discussing your phage-chromosome speculation. Most of this is already deeply engrained (as speculation) around here, but why does the site have to be a chromosome end? What kinds of experiments would bear on this?

A riverdice,

Joshua